Growth propagation of yeast in linear arrays of microfluidic chambers over many generations

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The growth of microorganisms is often confined in restricting geometries. In this work, we designed a device to study the growth propagation of budding yeast along linear arrays of microfluidic chambers. Vacuum assisted cell loading was used to seed cells of limited numbers in the up-most chambers of each linear array. Once loaded, cells grow until confluent and then overgrow, pushing some of the newborns into the neighboring downstream chamber through connection channels. Such a scenario repeats sequentially along the whole linear chamber arrays. We observed that the propagation speed of yeast population along the linear arrays was strongly channel geometry dependent. When the connection channel is narrow and long, the amount of cells delivered into the downstream chamber is small so that cells grow over several generations in the same chamber before passing into the next chamber. Consequently, a population growth of more than 50 generations could be observed along a single linear array. We also provided a mathematical model to quantitatively interpret the observed growth dynamics. © 2011 American Institute of Physics. [doi:10.1063/1.3668243]

I. INTRODUCTION

In responding to the environmental conditions and depending on their intrinsic fitness, microorganisms are able to live in different habitat types.^{1,2} Of particular interest is the guided growth of cells in restricting geometries and the propagation of a population from one habitat to another through connection channels. On the other hand, the use of restricting geometries allows tracking the growth dynamics at single cell level over a number of generations, which is important in determining the population phenotype of cells or organisms.³ Although the observation of the growth and propagation in conventional microbiology is trivial, only the recently developed microdevice technologies allow performing designed systematic analyses.^{4–14} For example, in order to study the switch-like behaviour of bacterial persistence, Balaban *et al.*⁶ used microfluidic channels to force *Escherichia coli* to grow along narrow channels derived from a single cell. In their device, the width and the height of the channels are comparable to the size of bacteria, allowing a guided growth and single cell observation which facilitates the follow up of lineages among the cells. However, the number of cell division which could be produced in a linear channel is limited to a few generations. Another example is the study of the spatial distribution of filamentous bacteria in a linear array of coupled microfluidic chambers. By using a linear array of microfluidic chambers, Keymer *et al.*¹² found an adaptive

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dynamics of *E. coli* sub-populations due to local extinction and colonization processes. Investigations have also been performed on non-filamentous microbes such as yeast in restricting geometries but most of them were focused on single cells or small populations of cells.^{15–18} For example, Rowat *et al.*¹⁸ cultured *Saccharomyces cerevisiae* in parallel linear channels and observed a correlation between growth phenotypes and age of cells over several generations. Cookson *et al.*¹⁹ devised a microchemostat for long-term acquisition of single-cell trajectories of yeast. The height of their imaging chamber is only 4 μm , which forced the cells to grow in a monolayer to facilitate single cell tracking. However, with their device configuration, most of new-borns of yeast could be washed away and only a few generations could remain in the observation zone. Similarly, Bennett *et al.*⁹ proposed a variable pressurization system to dynamically control the carbon source of the system and examine the transcriptional response of the glucose–galactose switch in yeast.

In this work, we show another prototype device which can be used for observation of cell growth over more than 50 generations. In our device, linear arrays of microfluidic chambers with interconnection channels of different lengths and sizes were designed and fabricated by two-level soft lithography. With the help of vacuum assisted self-loading, budding yeast cells were introduced into the up-most chambers of the linear arrays. Once reached confluent, cells overgrew, pushing some of the new-borns into the downstream chambers through the connection channels. When these channels are narrow and long, only a small amount of cells could be delivered sequentially into the neighboring downstream chambers. Consequently, the time needed for the population growth to occupy the whole habitat of the new chamber is relatively long and the sequential propagation along the whole linear array may take several days. Thus, one should be able to follow the cell division and the population growth along the linear array over a large number of generations. For illustration, we have then monitored the growth behaviours in the linear arrays with connection channels of different geometries. A mathematic model has also been introduced to interpret the observed growth behaviours.

II. EXPERIMENTAL

A. Device preparation

Figures 1(a) and 1(b) show, respectively, a schematic view and layout of the device used in the work, which consists of linear arrays of culture chambers, connection channels, diffusion nozzles (all in blue), a U shaped nutrient line (red), and a cell loading channel (green). The size of culture chambers is $50 \times 50 \mu\text{m}^2$. The width and length of connection channels are, respectively, in the range of 6–12 μm and 20–80 μm . The width and length of diffusion nozzles are, respectively, in the range of 3–5 μm and 20–30 μm . The number of the nozzles connected to a single chamber can be varied in the range from 1 to 5. To achieve a single cell observation, the height of these elements is limited to 5 μm . The width and height of nutrient and cell loading channels are 20 μm to ensure a relatively low flow resistance.

Our device was fabricated by standard soft lithography with a two-level mould (Fig. 1(c)). First, the mould was obtained using photolithography, lift-off, and reactive ion etching techniques. The culture chambers, the connection channels, and the diffusion nozzles were patterned into a thin layer of photoresist (AZ5214, Microchem) spin coated on a silicon wafer (Siliconx, France). After development and lift-off with a 50 nm thin film of nickel deposited by electron beam evaporation, the resist pattern was transferred into the silicon wafer by reactive ion etching with a SF_6 and CHF_3 gas mixture, at a pressure of 10 mT and a RF power of 10 W for 22 min. Then, a second layer of 20 μm thick photoresist (SU8-3025, Clariant Co.) was spin coated on the etched silicon wafer. Both nutrient and cell seeding channels were patterned using a mask aligner (SUSS MicroTec MJB4) with an alignment accuracy of about 1 μm . Finally, the silicon-SU8 mould was exposed to trimethylchlorosilane (TMCS) vapour for 3 min to facilitate the mould release.

The mould pattern was replicated into polydimethylsiloxane (PDMS) by casting. A and B components of PDMS RTV 615 (GE, France) were mixed at a ratio of 10:1 by weight and cast on the mould for a thickness about 5 mm and then kept in 80 °C for 2 h. After peeled off from

FIG. 1. Schematic illustration (a), layout (b), and fabrication process (c) of the device for investigating the growth propagation of yeast in linear arrays of micro-chambers. Cells are first loaded into the up-most chambers and then grow sequentially along the linear arrays (blue). Nutrient is provided by diffusion from a multi-U shaped channel (red). The first level of the mold for soft lithography is defined by photolithography, lift-off, and reactive ion etching techniques on the surface of a silicon wafer. The second level is patterned by photolithography with a SU8 resist. After molding by soft lithography, the PDMS layer is irreversibly bonded to a cover glass.

the mould, holes were punched to define inlets and outlets of cell seeding and nutrient channels in the PDMS layer with metal pipes and the PDMS layer was finally bonded to a cover glass. Before bonding, the surfaces of both PDMS and cover glass were treated with oxygen plasma for 1 min. The device was then kept in 80 °C for 1 h in order to achieve an irreversible bonding. Before used for cell culture, the device was washed with ultrapure water and sterilized in an autoclave for 20 min in an aluminium canteen. Afterward, the device was baked at 80 °C overnight to ensure the hydrophobicity of the PDMS surface. Finally, the device was put in the sterile Petri dish and placed in a vacuum of 100 mTorr for 10 min for degassing just before cell load experiments.

B. Cell preparation, loading, and culture

The yeast strain YCT50 (MATa *his3Δ met3Δ ura3Δ leu2Δ ura* MYO1::MYO1-GFP::HIS3 [pGREG506 ADH1pr-MCM-mCherry]) was plated onto agar plate of culture medium SC His⁻ Ura⁻. Before each experiment, a single colony was picked and suspended in 5 ml medium (SC His⁻ Ura⁻), shook at 30 °C overnight with a rotation speed of 200 rpm, diluted in fresh medium to about 10⁵ cells/ml, and grown overnight to about 10⁷ cells/ml. After centrifugation, cells were re-suspended in the medium at the density of 1 × 10⁶ cells/ml.

Vacuum assisted cell loading was used to place a limited number of yeast cells into the up-most chambers of each linear array.²⁰ To ensure that only a few cells could be loaded in the up-most chambers, the degassed PDMS device was first filled with culture medium. Then, the yeast suspension was injected into the top channel for 1 min and the channel was immediately washed with phosphate buffered saline (PBS) at flow rate of 10 μl/min for 1 min. Although the number of cells initially loaded into up-most chambers cannot be controlled exactly, one can still follow

their growth by optical imaging and begin the counting as soon as a single cell is loaded into the next chamber. After cell loading, culture medium was continuously injected into the multiple-U shaped channel at flow rate of $20 \mu\text{l/h}$ and the device was kept at a temperature between 27 and 30°C . Since our device is diffusion based,^{20,21} such a high flow rate in the nutrient channel will not introduce any shear stress in the chambers. Moreover, the length of the diffusion nozzles between the U-channel and the culture chambers was kept to be sufficiently small to ensure saturated nutrient conditions. Indeed, we did not observe any significant difference in growth rate between chambers having different numbers of diffusion nozzles. Note also that even though all culture chambers share one nutrient channel, the waste generated by the cells in upstream will not significantly affect cells in downstream because of the high flow rate of the nutrient solution.

C. Characterization and observation

Once the top-most chambers are fully occupied, cells penetrate into the next chamber for a sequential growth through the connection channel. Then, the growth will be continued until the whole linear arrays are occupied. The cell growth behaviours could be monitored with a microscopy (Olympus IX71, Japan) equipped with an automated scanning stage in a time-lapse and multi-location mode (Andor, UK). Green and red fluorescent images were obtained with 488 and 561 nm laser excitations with 525/50 and 605/20 nm single band fluorescence filter, respectively. Time-lapse images were recorded by an Electron Multiplying Charge Coupled Device (EMCCD) camera (Andor, UK). Cell numbers in differential interference contrast (DIC) images were counted in a semi-automatic way using custom software. Numerical calculations were performed using MATLAB programs (Mathworks, US).

III. RESULTS AND DISCUSSION

Before going into details of the analyses, we show some general features of the population growth of yeast cells in a liner array of coupled chambers. Fig. 2 shows DIC (a) and fluorescence (b and c) images of three culture chambers where the growth propagation front can be clearly seen in the last (bottom most) channel. Here, the width and length of connection channels are 6 and $80 \mu\text{m}$, respectively. The images were taken after 3 days culture at the location of 4th to 6th chambers. Clearly, the cell densities in the three chambers are different from each other and well-resolved single layer imaging can only be possible with the leading chamber just behind the propagation front. It seems that the yeast cells in the two other chambers are highly compact due to growth pressure, resulting in a deformation of the culture chamber due to low Young's module of PDMS. The same culture behaviour could also be found with other channel geometries.

The green fluorescence image in Fig. 2(b) was resulted from protein MYO1-GFP which is an indication of yeast productivity. In fact, MYO1-GFP is localized at the presumptive bud site and remains at the mother-bud neck until cytokinesis is completed.²² Apparently, all cells were still productive during observation no matter how the yeast population is compacted. We will show that the yeast population in a culture chamber can continuously grow against the PDMS walls before reaching a critical value of growth pressure. The red fluorescence image in Fig. 2(c) was due to protein MCM-mCherry which is localized in the nuclei during the G1 phase of the cell cycle of yeast and spread out in other phases.²³ As can be seen, cells were in different phases but more elder cells seem to be in G1 phase.

A. Cell growth in leading chambers

We first discuss the yeast growth in a single chamber. A sequence of DIC images recorded each 15 min from the same culture chamber with connection channels of $6 \mu\text{m}$ wide and $40 \mu\text{m}$ long (Fig. s1)²⁴ allowed us to count the variation of the cell number as a function of culture time. At the beginning, a few cells were already located in the chamber but they were in a quiescent phase since they did not divide later on. At 30 min, a few cells appeared at the entrance of the chamber coming through the connection channel due to division and mechanic push from the yeast in the upstream chambers. Then, the cell number increased with the

FIG. 2. Yeast growth in a linear array of $50 \times 50 \mu\text{m}^2$ square chambers with $6 \mu\text{m}$ wide and $80 \mu\text{m}$ long connection channels. (a) DIC image of the last three occupied chambers. (b) Corresponding fluorescence image of MYO1-GFP protein. (c) Corresponding fluorescence image of MCM-mCherry protein.

increase of culture time until the whole habitat was occupied. Note, however, that, the increase of the cell number was not homogenous since a small number of cells emerged during the time interval between 135–150 min, 180–195 min, and 210–225 min, respectively. Once the whole chamber was occupied, the division continued, leading to an overgrowth in the chamber area on one hand and the invasion of the downstream chambers on the other hand. As can be seen from the image sequence of Fig. s1,²⁴ single cell layer can be well-resolved so that it allows accurate cell number counting. The variation of the cell number in the culture chamber is then plotted in Fig. 3 for a growth period of 7 h until confluent (square markers). The solid curve of the figure was obtained based on the following theoretical model.

Considering a culture condition of rich nutrient but limited space, the following equation can be derived:²⁵

$$\frac{dN_i}{dt} = k_i N_i - \beta N_i N, \quad (1)$$

where N_i is the cell number originated from the one deliberated from the connection channel at time t_i , i is the index of the cell seeding events, k_i and β are, respectively, the specific growth rate and a coefficient. N is the total cell number in the culture chamber at time t , given by

$$N = \sum_{i=1}^M H(t - t_i) N_i, \quad (2)$$

where H is the step function, i.e., $H(x) = 1$ if $x \geq 0$ or $H(x) = 0$ if $x < 0$. M is the total number of cell delivery events. For simplicity, we will make no distinction on the growth rate by setting $k_i = k$ for all cells.

In the case of single cell seeding, Eq. (1) has the following well-known solution

$$N(t) = \frac{k/\beta}{1 + (k/\beta - 1)e^{-kt}}. \quad (3)$$

Otherwise, Eq. (1) can be solved numerically for a sequential cell seeding. The solid curve in Fig. 3 was obtained with doubling time $t_d = \ln 2/k = 80$ min, $\beta = 1/155 \text{ min}^{-1}$ and a total cell seeding number of 35. To take into account the age of the seeding cells, a half doubling time was used for their first cell division period. For comparison, the growth curve calculated with a single cell seeding was also plotted in Fig. 3 (dashed line), which obviously failed to obtain a good fitting for the experimental data.

B. Cell passage through connection channels

The cell passage through connection channels is strongly channel geometry dependent. Indeed, the overgrowth of yeast in the upstream chamber and the cell division inside the connection channels both contribute to the displacement of leading cells and the deformation of the PDMS device. With the increase of the cell number in the new chamber, the pressure gradient in the connection channel decreases so that it becomes more difficult to push cells into the chamber and the cell seeding speed should not be constant. Experimentally, we observed not only a one-by-one sequential cell loading but also grouped loading events. This phenomenon is more frequent in the case of wider and shorter channels. Fig. s2 shows DIC images of a typical seeding and growth sequence of yeast in a chamber with connection channels of $12 \mu\text{m}$ width and $20 \mu\text{m}$ length, taken each 15 min for a period of 3 h. Here, the time needed for the whole chamber occupation is much shorter than that with narrower and longer connection channels because of much increased cell loading rate. As can be seen from these well-resolved images of single cell layers, the cell number in the culture chamber increases rapidly in the time intervals of 60–75, 90–105, and 105–120 min. The variation of the cell number in the culture chamber is plotted in Fig. 4 (square markers). The solid line in the same figure was obtained by calculation taking into account the observed loading irregularity. Here, in addition to the regular loading of 8 cells over the whole growth period, 28 and 30 cells were added at about 15 and 75 min, respectively. For

FIG. 3. Variation of the cell number as a function of culture time in one of the micro-chambers. Cells are sub-sequentially loaded into the chamber from a $6 \mu\text{m}$ wide and $40 \mu\text{m}$ long connection channel. The solid line is a fitting of the experimental data with a homogenous loading of 28 cells during 7 h. The dashed line is obtained with only one cell input.

FIG. 4. Variation of the cell number as a function of culture time in a chamber with $12\ \mu\text{m}$ wide and $20\ \mu\text{m}$ long inter-connection channels. The solid line is a fitting with a homogeneous loading of 8 cells and two additional loading of 28 cells at 13 min and 30 cells at 75 min. The dashed line is obtained with a homogeneous cell loading after an initial loading of 28 cells at 13 min.

the sake of simplicity, this calculation was done without considering the nonlinear term ($\beta = 0\ \text{min}^{-1}$). For comparison, the growth curve calculated by using an initial loading of 28 cells followed by a regular loading of 30 cells is also shown in Fig. 4 (dashed line).

C. Growth propagation by sequential chamber occupation

Following the above analyses, the growth propagation of yeast in the linear arrays of coupled micro-chambers should also depend on the geometry of connection channels. Fig. 5 shows the number of occupied chambers as a function of culture time for the linear arrays with connection channels of widths 6 and $12\ \mu\text{m}$ and lengths 20, 40, and $80\ \mu\text{m}$. Clearly, the growth propagation speed in the linear array with $12\ \mu\text{m}$ wide and $20\ \mu\text{m}$ long connection channels (8–10 chambers per day) is much higher than that with $6\ \mu\text{m}$ wide and $80\ \mu\text{m}$ long channels (2 chambers per day in average). Other channel geometries showed a propagation speed of 4–5 chambers per day. We also measured cell sizes of new-borns but did not find noticeable channel size dependence. The main interest of using the proposed linear culture chamber arrays relies on its ability of guiding a sequential growth over a large cell generations in the same device. Comparing to the more conventional setup where only a limited cell generation could be observed with one monolayer cell colony, our device overcomes this limitation by introducing a number of culture chambers which can effectively retard the growth propagation. Assuming that the last generation is always in the front of the growing population in our device and 4–5 cell generations per culture chamber, a total cell generation of about 80–100 can be expected for a line array of 20 chambers. As shown in Figs. 2, s1, and s2,²⁴ it was possible to track single cells in the foremost chambers, thereby providing a way to monitor a sequential growth at single-cell level over a large number of generations. Since our investigation was mainly focused on the population growth along the linear chamber arrays, no detailed analyses has been done on single cell tracking in this work. More powerful tools for image processing and data handling would be required for such a single cell tracking over many generations. Finally, the single cell tracking in all individual chambers would be possible, providing well-resolved single cell layers in cell chambers. To this end, the channel size of the nutrient diffusion nozzles has to be enlarged to allow yeast partially escaped from the overgrown chambers.

D. Growth pressure induced device deformation

As mentioned above, cells overgrow in the PDMS chamber after the whole habitat is occupied because of the growth pressure and the low Young's module of PDMS ($\sim 1\ \text{MPa}$).²⁶

FIG. 5. Variation of the number of occupied chambers as a function of culture time for linear arrays of the same chamber size but different geometries of connection channels.

Comparing to PDMS, the Young's module of the cell wall of budding yeast is 100 times higher.²⁷ Therefore, the deformation of yeast should be much smaller than that of PDMS. We observed that the PDMS chamber is largely deformed until a certain threshold strain. Measuring the deformation of the PDMS chamber should allow us estimating the critical value of the growth pressure under which cells stop to grow. Experimentally, we observed about 15% increase of the channel width in the 4th chamber behind the first one and no significant increase for those beyond. Considering $\sigma = E\epsilon$, where σ , ϵ , and E are, respectively, the normal stress, the strain, and the Young's module of PDMS, we obtained a normal stress of about 0.15 MPa for 15% strain of the PDMS chamber. Previously, the mechanic forces of fission yeast have been studied by analyzing the single cell growth against the PDMS walls,²⁸ showing an internal turgor pressure of about 0.85 MPa, which is significant different from that we have estimated from the budding yeast population. More systematic investigations are needed to clarify the growth pressure difference between the two types of yeast as well as that between a single cell and a cell population.

The overgrowth of yeast in a PDMS chamber results also in a change of the chamber height. Following the recent work of Hardy *et al.*,²⁹ the maximum change in the chamber height can be expressed by

$$\Delta \bar{h} = \gamma \rho W / E, \quad (4)$$

where W is the chamber size, E is the Young's module of PDMS, p is the pressure, and γ a proportional constant which depends on the channel geometry and mechanical properties. For a PDMS cover layer of 5 mm thickness above the culture chamber, $\gamma = 0.72$,²⁹ which results in a

maximum change in chamber height about $5.5\text{ }\mu\text{m}$. This meant that at least two yeast layers were grown in the centre of fully occupied culture chamber. Indeed, the defocused images of the chambers behind the leading chamber verified such a prediction. Finally, we noticed an aggressive penetration of yeast cells into the nutrient diffusion lines which are connected to the overgrown chambers. Since the width of the diffusion channels is at least twice smaller than the size of the yeast, their penetration provided another evidence of the important growth pressure of the sub-population in PDMS chambers.

IV. CONCLUSION

We have proposed a new device configuration to culture and observe growth and propagation of yeast in restricting geometries over a large number of generations. We found that the population of yeast could grow and propagate along linear arrays of micro-chambers with narrow interconnection channels. A mathematic model has been used to quantitatively interpret the growth and propagation behaviours of the yeast population in such a designed geometry. In addition, we deduced the growth pressure of budding yeast in a confined system. Finally, the device configuration we proposed is simple and versatile which should be applicable for a number of investigations such as growth dynamics and phenotype variability of bacteria as well as invasive growth of cancer cells.

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- ¹L. Ranjard and A. S. Richaume, *Res. Microbiol.* **152**, 707 (2001).
- ²J. Männik, R. Driessen, P. Galajda, J. E. Keymer, and C. Dekker, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14861 (2009).
- ³M. R. Bennett and J. Hasty, *Nat. Rev. Genet.* **10**, 628 (2009).
- ⁴A. Groisman, C. Lobo, H. J. Cho, J. K. Campbell, Y. S. Dufour, A. M. Stevens, and A. Levchenko, *Nat. Meth.* **2**, 685 (2005).
- ⁵S. Park, P. M. Wolanin, E. A. Yuzbashyan, H. Lin, N. C. Darnton, J. B. Stock, P. Silberzan, and R. Austin, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13910 (2003).
- ⁶N. Q. Balaban, J. Merrin, R. Chait, L. Kowalik, and S. Leibler, *Science* **305**, 1622 (2004).
- ⁷F. K. Balagaddé, L. C. You, C. L. Hansen, F. H. Arnold, and S. R. Quake, *Science* **309**, 137 (2005).
- ⁸W. T. Liu and L. Zhu, *Trends Biotechnol.* **23**, 174 (2005).
- ⁹M. R. Bennett, W. L. Pang, N. A. Ostroff, B. L. Baumgartner, S. Nayak, L. S. Tsimring, and J. Hasty, *Nature* **454**, 1119 (2008).
- ¹⁰Y. Taniguchi, P. J. Choi, G. W. Li, H. Y. Chen, M. Babu, J. Hearn, A. Emili, and X. S. Xie, *Science* **329**, 533 (2010).
- ¹¹X. J. Luo, K. Y. Shen, C. X. Luo, H. Ji, Q. Ouyang, and Y. Chen, *Biomed. Microdevices* **12**, 499 (2010).
- ¹²J. E. Keymer, P. Galajda, C. Muldoon, S. Park, and R. H. Austin, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 17290 (2006).
- ¹³J. E. Keymer, P. Galajda, G. Lambert, D. Liao, and R. H. Austin, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 20269 (2008).
- ¹⁴R. H. Austin, C. K. Tung, G. Lambert, D. Liao, and X. Q. Gong, *Chem. Soc. Rev.* **39**, 1049 (2010).
- ¹⁵C. R. Terenna, T. Makushok, G. Velze-Casquillas, D. Baigl, Y. Chen, M. Bornens, A. Paoletti, M. Piel, and P. T. Tran, *Curr. Biol.* **18**, 1748 (2008).
- ¹⁶L. Robert, G. Paul, Y. Chen, F. Taddei, D. Baigl, and A. B. Lindner, *Mol. Syst. Biol.* **6**, 357 (2010).
- ¹⁷C. J. Murakami, C. R. Burtner, B. K. Kennedy, and M. Kaeberlein, *J. Gerontol. A* **63**, 113 (2008).
- ¹⁸A. C. Rowat, J. C. Bird, J. J. Agresti, O. J. Rando, and D. A. Weitz, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 18149 (2009).
- ¹⁹S. Cookson, N. Ostroff, W. L. Pang, D. Volfson, and J. Hasty, *Mol. Syst. Biol.* **1** (2005).
- ²⁰L. Wang, X. F. Ni, C. X. Luo, Z. L. Zhang, D. W. Pang, and Y. Chen, *Biomed. Microdevices* **11**, 679 (2009).
- ²¹X. Li, L. Liu, L. Wang, K.-i. Kamei, Q. Yuan, F. Zhang, J. Shi, A. Kusumi, M. Xie, Z. Zhao, and Y. Chen, *Lab Chip* **11**, 2612 (2011).
- ²²E. Bi, P. Maddox, D. J. Lew, E. D. Salmon, J. N. McMillan, E. Yeh, and J. R. Pringle, *J. Cell. Biol.* **142**, 1301 (1998).
- ²³H. Yan, A. M. Merchant, and B. K. Tye, *Genes Dev.* **7**, 2149 (1993).
- ²⁴See supplementary material at <http://dx.doi.org/10.1063/1.3668243> for supporting figures.
- ²⁵D. Tilman and J. A. Downing, *Nature* **367**, 363 (1994).
- ²⁶W. O. J. C. Lötters, P. H. Veltink, and P. Bergveld, *J. Micromech. Microeng.* **7**, 145 (1997).
- ²⁷A. E. Smith, Z. B. Zhang, C. R. Thomas, K. E. Moxham, and A. P. J. Middelberg, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 9871 (2000).
- ²⁸N. Minc, A. Boudaoud, and F. Chang, *Curr. Biol.* **19**, 1096 (2009).
- ²⁹B. S. Hardy, K. Uechi, J. Zhen, and H. P. Kavehpour, *Lab Chip* **9**, 935 (2009).